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Biological Approaches to Nematode Management in Nursery Soils

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ABSTRACT:

This study was part of a larger project dealing with developing sustainable alternatives to methyl bromide (MBr) use, with particular emphasis on the effects and appropriate use of a mustard type of biofumigants against the northern root-knot nematode (RKN, *Meloidogyne hapla*). This, in turn, requires a clear relationship between nematode biology (life cycle) and the purpose (as a trap crop or as a green manure) for which mustard type biofumigants are to be used. RKN is one of the three economically significant plant-parasitic nematode genera in Michigan agriculture. In the absence of resistant cultivars and impending loss of MBr with few sustainable alternatives, the nursery and vegetable industries face significant challenges in managing RKN. Jointly funded by MDA, MPIC and Celery Research Inc., this study tested the interactions of oil seed radish cv. 'Common' (OSRC), soil types and selected Michigan RKN populations from nursery (PN, WL and SW) and vegetable (ED) soils. Isolated from sandy (PN and WL), loamy sand (SW) and muck (ED) soils, the nematodes had exhibited reproductive potential (pathogenecity) differences in tomato, suitable host for RKN but had never been planted in the fields from where the nematodes came. Thus, raising the question of whether or not the one-option-fits-all management approach applies to these nematodes. When the PN, WL, SW and ED populations were inoculated at 2,000 to 3,000 eggs/300 cm^3 of either sandy (WL), loamy sand and muck soils brought from the same fields as the nematodes and maintained for 500 degree days (DD, base 10 ^oC), generally similar numbers of juveniles and adults were found in OSRC roots. This shows the approximate time when the nematodes complete a life cycle and that OSRC can be a tool against these RKN populations, but as a trap crop by destroying the plants before the end of the nematode life cycle. The infection rate of the nematodes however was significantly higher in sandy soil, showing that the efficacy of OSRC against these RKN populations is dependent on soil type. Thus, challenging the one-option-fits-all management approach. Overall, the study provides the necessary basis for comprehensive field studies to develop a management model for biofumigant use for the prevailing Michigan conditions.

INTRODUCTION

The northern root-knot nematode (RKN, *Meloidogyne hapla*), root-lesion (RLN, *Pratylenchus* spp.), and cyst (*Heterodera* spp.) are the most problematic nematodes in the diversified Michigan agriculture (Bird et al., 2004). Without nematode resistant cultivars and the gradual phase out and/or restrictions of broad-spectrum nematicides, many commodities, the nursery and vegetable industries in particular, face short- and long-term challenges in managing nematodes. Research priorities recognized by MNLA and the vegetable industry stakeholders include mapping of nematode populations, breeding for nematode resistance, developing alternatives to broad-spectrum pesticides and nematicides, identifying effective cultural practices, biosuppression, nematicidal plants, and effective and sustainable soil amendments (http://www.greeen.msu.edu/priorities.htm).

Meeting the stakeholders' multi-dimensional and short-, mid- (most), and long-term (breeding for resistance) priorities hinge on understanding the complexities of the nematodes and the production systems in question. For example, RKN has a high degree of genetic (Chen et al. 2003; Liu and Williamson, 2004) and parasitic variability even in populations that come from about 20 miles apart (Melakeberhan et al. 2006a). Although most ideal, developing management practices based on each nematodes' interaction with its hosts will be expensive. A worthwhile strategy, within the umbrella of industry priorities, is to design practices that affect multiple crops and/or nematodes. Using an RKN model, this project was developed to look at the effects of mustard type biofumigants on nematode populations. The objective is to test the efficacy of oilseed radish (OSR) on RKN populations in muck, loamy sand and sandy soils under greenhouse conditions.

MATERIALS AND METHODS

Greenhouse conditions:

Greenhouse studies were conducted to determine the interactions among OSR, soil types and RKN populations from the different soil types. Greenhouse conditions were set at 28 ± 2 ^{o}C with diurnal cycles of 8 hours dark and 16 hours day with photosynthetically active radiation of 450 to 550 µmol.s-^{1}.m-^{2} at canopy level. All studies were conducted using 300 cm^{3} of steam-sterilized soil contained in white Styrofoam cups. Pots were watered to saturation with tap water one hour prior to transplanting and as needed thereafter.

Nematode sources and soil types:

The nematodes used in the study included two populations from sandy (PN and WL) and one each from loamy sand (SW) and muck (ED) soil fields with pHs of 6.56, 7.43, 7.15 and 6.30, respectively (Melakeberhan et al., 2006a). The muck soil was a vegetable production system, the rest were nursery fields, and all were within a 20 miles radius. Tested in tomato (*Lycopersicon esculentum* L.) cv 'Rutgers', neutral host and not planted in the fields before, the nematodes showed differences in pathogenicity (Melakeberhan et al., 2006a). Consequently, challenging the one-option-fits-all management approach.

The three soil types, sandy, loamy sand, and muck, used in the study were brought from the fields where the WL, SW, and ED nematodes were isolated, respectively. This was necessary to test the nematodes' behavior in relation to OSR response under the soil types where they had been residing before they came to the greenhouse conditions. Barrels of soils from each field were brought to campus during the summer of 2005 and steam-sterilized prior to being used in the study.

Experiments:

In the first study, one-week-old OSR cultivar cv 'Common' (OSRC) and an organically certified stoke (OSRS, Lot # RO4S-PSOSR, R. Stuckey, North Alger Rd., Alma, MI, 48801) seedlings were transplanted from potting soil into 300 cm^3 Styrofoam cups containing the assigned soil type. Three days after transplanting, seedlings of the two OSR cultivars were inoculated with either 0 or 3,000 eggs of the ED, PN and SW populations per pot (Mennan et al., 2006). Inocula were collected from greenhouse cultures following Hussey and Barker's (1973) bleach (5% NaOCl) method. A total of 96 experimental units (4 nems x 3 soils x 2 hosts x 4 reps) were used. The experiment was terminated 28 days after nematode inoculation.

In the second study, delayed about six months due to some difficulties with nematode cultures, only OSRC was used (see results). The four nematodes (PN, WL, SW and ED) and a control were inoculated at 0 or 2,000 eggs to 3-week-old seedlings, and the experiment lasted 30 days after inoculation. A total of 60 experimental units (5 nems x 3 soils x 4 reps) were used.

At the experimental temperature, 504 degree-days (DD, base 10 ^oC) and 540 DD were accumulated by the end of the first and second studies, respectively. These are enough heat units under which all nematodes can complete a life cycle (Insera et al., 1982).

Nematode infection measurements and data analysis:

At the end of the studies, shoots were cut off at the base and fresh weights determined. Roots were very carefully separated from soil, gently washed free of soil, and rated for root-knot galling indices on a 0 (no galling) to 5 (more than 75 % of the root system galled) scale (Kinloch, 1990). In order to minimize experimental errors, whole root systems were stained and nematodes counted (Melakeberhan and Dey, 2003). Stained samples were kept at 4^o C until counted, *M.*

hapla developmental stages determined as illustrated in Agrios (1997), and categorized as second-stage juveniles (J2), third and fourth-stage males and females (J3/J4) and females (Melakeberhan and Dey, 2003). Nematode population density data were standardized on a per g fresh root weight basis and statistical analyses were performed with SAS System Release 8 (SAS Institute, Cary, NC, 2000).

Results and Discussion

Mustard types of crops can be used as trap or cover crops or as biofumigants when incorporated into the soil to suppress weeds, nematodes and other soil borne yield-limiting biological factors (Hafez and Sundararaj, 2001; McSorley et al., 1997; Ngouajio and Mutch, 2004; Tseo et al., 2002). In order to exploit the multipurpose use of biofumigants, we need to understand the intricate relationships among nematode biology, their living environments, and the performance of the biofumigants. Integrating nematode biology and parasitic variability with soil environment, this study provides biological basis towards the appropriate use of OSR for Michigan conditions.

The effect of soil type was most striking. Across nematodes, sandy soil was the most (P = 0.05) favorable for nematode invasion of OSR (Fig. 1, Table 1). Thus, clearly showing that the best use of OSR will be dependent on the soil type. Subsequently, challenging the one-option-fits-all management approach. For example, high RKN infection of OSR in sandy soil can be good if the plants are removed before the nematodes complete a life cycle. Otherwise, it will increase the RKN problem.

The nematodes used in this study came from nursery and vegetable production systems within approximately 20 miles radius. They have been shown to exhibit parasitic variability and reproductive potential differences (Melakeberhan et al. 2006a). By definition, a difference in

reproductive potential can lead to differences in threshold levels. Consequently, the management decision-making processes with significant economic and/or ecological implications to growers and the environment.

In the first experiment, the SW population was most ($P = 0.05$) pathogenic while al four nematode populations infected similarly in Experiment 2 (Table 2). Although nematodes in whole root systems were counted, the difference in nematode numbers may have natural experimental errors. Nonetheless, the results show that all four RKN populations do infect OSRC. Moreover, the presence of juveniles and adult stages shows that the nematodes can reproduce in OSRC.

Infection by these nematodes can be good or bad depending on the purpose the OSR use. For example, if the OSRC is to be used as a trap crop for RKN it is good that more nematodes invade the roots. The nematodes however should not be allowed to complete a generation, which means that the OSR in use has to be removed or chopped and incorporated into the soil. This, in turn, requires knowing when to terminate the controlled or field experiment. All other things being similar, temperature is the best indicator for determining when the nematode is likely to complete its life cycle. Based on this study and using eggs as inoculum, approximately 450 to 500 DD from the time of inoculation appears to be the likely time for the nematodes to complete a life cycle. However, comprehensive field studies are needed to determine if the controlled experimental results can be reproduced under field conditions.

The OSR cultivars tested were selected for agronomic and availability reasons. Although galling was more in OSRC than in OSRS, the data show that both hosts carried similar numbers of nematodes (Fig. 1). Hence, which OSR to use may depend on cost and availability. The relationship among suitability to nematodes, soil type and OSR growth (Table, 3) sheds some

light to the purpose of OSR use. As discussed here, sandy soil favors nematode infection of OSRC while vegetative mass growth may be more in the muck and loamy sand soils. Thus, the removal of RKN when using OSRC as a trap crop needs to be balanced with the potential use of OSRC as a biofumigant when incorporated into the soil.

Multi-taxa nematode presence in any given environment is likely and presents additional challenges on how OSR (and other tactics) may be used. When considering OSR against RKN in the presence of other nematodes, integrating the purpose (form) OSR use and nematode biology become critical. For example, RKN (sedentary endo-parasite) has only one infective stage (second-stage juvenile), whereas, all four vermiform stages of RLN (migratory endo-parasite) are infective. If OSR is to be used as a trap crop for either RKN or RLN, it needs to be removed or chopped before the nematodes complete their life cycle. When incorporated into the soil, OSR serves as a biofumigant against RKN, RLN and other nematodes present in the soil. Because RKN has only one infective stage, suppressing its population density with trap cropping may be easier than RLN. Again, field tests are needed to verify these results.

Overall, the study shows the significance of incorporating nematode biology, parasitic variability, and soil conditions to provide valuable information towards making decisions when considering the best use OSR. Most significantly, it is fair to say that the one-option-fits-all management approach needs to be reconsidered. Further laying the groundwork for adopting site-specific management approaches (Melakeberhan, 2002) as well as incorporating new and emerging more efficient trap crops for RKN (Melakeberhan et al., 2006b).

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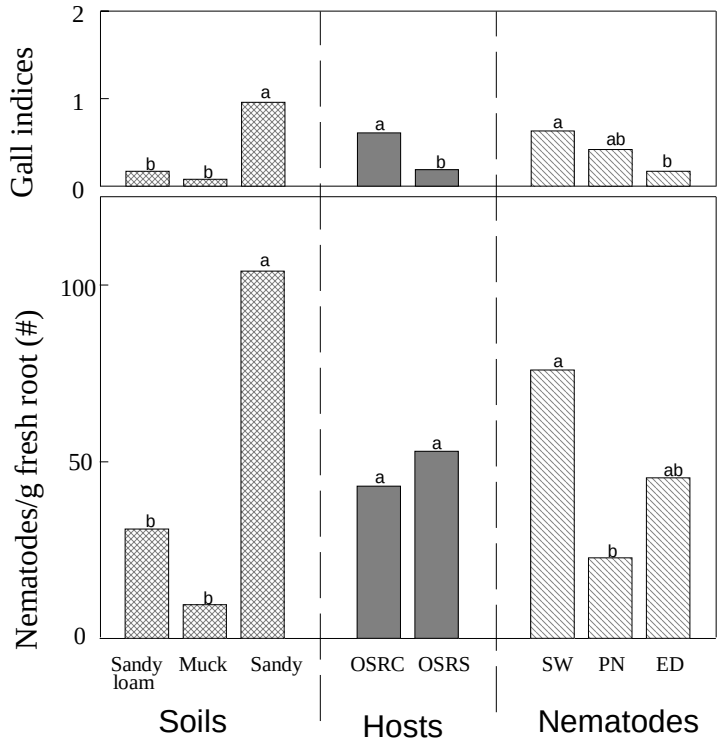

Fig. 1. The effects of soil types, oil seed radish cultivars (OSRC and OSRS), and three Michigan RKN populations (ED, PN and SW) on the numbers of nematodes per g fresh root and galling indices.

Gall indices are based on a 0 (none) to 5 (100%) scale.

Bars with the same letters within a category are not statistically different from one another.

Table 1. Numbers of juveniles (JUVS), adult females and total nematodes per gram of fresh root weight from loamy sand (LS), muck (M) and sandy (S) soils in Experiment 2.

Soil	JUVS	Females	Total
LS	4.1	83.6 b[1]	87.7 b[1]
M	25.4	72.1 b[1]	98.0 b[1]
S	16.4	293.9 a[1]	311.8 a[1]
P	*0.2984*	*0.0001*	*0.0001*

[1] Means followed by different letters with a category are statistically significant at the probability (*P*) levels indicated.

Table 2. Numbers of juveniles (JUVS), adult females and total nematodes per gram of fresh root weight of the *Meloidogyne hapla* populations (Mhp) from loamy sand (SW), Sandy (PN and WL) and muck (ED) soils, and the interactions of Mhp and soil in Experiment 2.

Mhp	JUVS[1]	Females[1]	Total[1]
SW	5.1	131.8	137.4
PN	10.0	149.9	160.4
ED	18.9	148.4	168.2
WL	25.5	182.1	208.5
P	*0.1076*	*0.3414*	*0.1754*
Mhp*soil	*0.2935*	*0.2828*	*0.2742*

[1] Means with a category are statistically significant at the probability (*P*) levels indicated.

Table 3. Effect of loamy sand (LS), muck (M) and sandy (S) soils, *Meloidogyne hapla* populations (Mhp) and their interactions on root and shoot fresh weight (g) in Experiment 2.

Soil	Root[1]	Shoot[1]
LS	2.16 a[1]	10.41 a[1]
M	0.97 c[1]	12.17 a[1]
S	1.51 b[1]	4.93 b[1]
P	*0.0064*	*0.0001*
Mhp	*0.5041*	*0.1444*
Mhp*soil	*0.9637*	*0.6619*

[1] Means with a category are statistically significant at the probability (*P*) levels indicated.